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Research Article

Combination of the Leaves of Annona muricata, Launaea taraxacifolia and Tridax procumbens: An Herbal Medicine for the Prevention and Treatment of Hypertension, Atherosclerosis and Cardiovascular Diseases

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Abstract

High blood pressure and cardiovascular diseases are the non communicable diseases that cause the most deaths in Africa. They affect the most disadvantaged sections of society who have no other choice but to treat themselves with plants. In the purpose of proposing to the populations an herbal medicine that can help prevent and even treat hypertension and cardiovascular diseases, we have studied the bioactive substances (secondary metabolites, vitamins and minerals) contained in three plants used by traditional healers to treat high blood pressure and cardiovascular diseases. Our results have shown that these plants together contain polyphenols, leucoanthocyanins, anthocyanins, triterpenoids, steroids, reducing compounds, as well as vitamins such as vitamins A, E, C, K1 and minerals like calcium, potassium, iron. These three plants together could constitute a dietary supplement whose regular intake would help prevent or treat high blood pressure, atherosclerosis and cardiovascular diseases.

Keywords: Atherosclerosis; Hypertension; Plants; Prevention; Treatment

Introduction

Cardiovascular disease (CVD) is now the major cause of death in the world. In 2015, they were responsible for 17.7 million deaths, or nearly one in three deaths worldwide that year. More than three-quarters of CVD-related deaths and 82% of early deaths before the age of 70 occur in low- and middle-income countries [1]. Cardiovascular disease refers to all diseases of the heart and arteries. They are caused in large part by deposits of cholesterol on the walls of the arteries. These deposits block the flow of blood to the heart, brain or legs, causing angina, heart attack and stroke. Among the cardiovascular diseases, the most frequent in Benin are: Arterial hypertension (HTA), estimated at 27.5%; Obliterations of the Arteries of the Lower Limbs, estimated at 3.9%; Cerebral Vascular Accidents (CVA) estimated at 4.6%; and Heart Failure at 1%; [2]. One of the causes of cardiovascular diseases is atherosclerosis. Atherosclerosis is a pathological process leading to thickening of the walls of the arteries. The deposition of cholesterol in the arterial wall triggers complex phenomena which lead to the formation of atheromatous plaques. This vulnerability of atherosclerotic plaque can progress more or less rapidly depending on the risk

factors of each individual. Narrowing of blocked arteries causes serious problems when the blood supply to the heart is reduced, leading to angina or heart attack, or to the brain, which can lead to a stroke [3]. Several factors are responsible for the development of atherosclerosis: dyslipidemia, type 2 diabetes, obesity, metabolic syndrome and high blood pressure. Arterial hypertension activates the renin-angiotensin system which induces oxidative stress via the activation of NAD (P) oxidase by the angiotensin 2 receptor AT1. This results in the production of superoxides which inactivate nitric acid and causes vasodilation of blood vessels [4]. Several studies have shown that inflammation also plays an important role in the initiation and progression of atherosclerosis. Patients with heart attacks and strokes have high levels of C reactive protein; also the use of anti-inflamatory drugs in the prevention of cardiovascular diseases is effective [5]. Thus oxidative stress and inflammation therefore play a very important role in the development of cardiovascular diseases [6,7]. In Africa up to 80% of the population uses traditional medicine for treatment. Many researchers are turning to the ethno pharmacological approach to find new active natural derivatives. Studies have focused on the beneficial effects and targets of medicinal plants to develop a scientific basis for the preventive and therapeutic use of these plants. Plants of the Asteraceae and Annonaceae family are often used by traditional healers. Asteraceae (*Launaea taraxacifolia and Tridax procumbens*) are used in the treatment of arterial hypertension, diabetes, liver diseases, malaria, bronchitis, conjunctivitis [8] and annonaceae (*Annona muricata*) in the treatment cardiovascular diseases, cancer, fever, also in the treatment of diarrhea and dysentery [9]. Several studies have demonstrated the biological activities of *Annona muricata* [10], *Launaea taraxacifolia* [11] and *Tridax procumbens* [12]. After a phytochemical analysis to know the secondary metabolites of these three plants, we determined their compositions in vitamins and minerals which could explain the antioxidant, anti-inflammatory and hypotensive activities to demonstrate that the combination of these three plants could form an herbal medicine able to prevent and treat high blood pressure, atherosclerosis and cardiovascular diseases.

Materials and Methods

Materials

The leaves of *Annona muricata, Launaea taraxacifolia* and *Tridax procumbens* were collected in "Dassa-zoumé" (city of center of Benin) from October to November 2019. The leaf samples were cleaned and dried at ambient temperature in our laboratory to better preserve molecules sensitive to heat and light. They were then crushed and bottled.

Methods

Phytochemical screening of the leaves of the three plants

Phytochemical screening is based on the differential reactions (coloring and precipitation) of the main groups of chemical compounds contained in plants according to the Houghton and Raman method [13].

Identification and determination of water-soluble and fat-soluble vitamins from the three plants by HPLC [14]

Identification and dosage of water-soluble vitamins (vitamin C)

The identification and determination of vitamin C is carried out by high performance liquid chromatography (HPLC).

Preparation of standard solutions

A standard solution of vitamin C is prepared at a concentration of 1 mg/ml in methanol. Three series of successive dilutions were subsequently carried out to obtain solutions of concentrations: 500 µg/mL, 250 µg/mL and 125 µg/ml. These dilutions constitute the range of concentrations used for calibration. A calibration line was obtained every day.

Extraction

0.5 g of sample is weighed and placed in 5 mL of an aqueous solution of 5% glacial acetic and 1% thiourea. This mixture is placed in an oven for 20 hours at 60 °, then 5 ml of 2% oxalic acid are added. The macerate is then filtered and its pH is readjusted to 4.5 by adding 1N NaOH. Finally, this solution is washed twice in a separating funnel with 10 ml of petroleum ether. The organic phase is rejected. The aqueous phase is kept then the pH is readjusted to 2.5 with acetic acid. Subsequently, 10µl of each sample prepared under the above conditions are injected for chromatographic analysis according to chromatographic conditions.

Identification and determination of fat-soluble vitamins (A, E. K1)

Preparation of standard solutions

A standard solution of vitamin (A, E, K1) at a concentration of 1 mg/ml in methanol was prepared (stock solution). 05 series of successive dilutions were subsequently carried out to obtain solutions of the following respective concentrations, 500µg/mL, 100 µg/mL, 20µg/mL, 4 µg/ml and 1 µg/mL. These dilutions constitute the range of concentrations used for calibration. A calibration line was obtained each day.

Extraction

25 mL of hexane are added to 0.5 g of plant drug contained in an Erlenmeyer flask. The solution is subjected to ultrasound for 30 minutes in an ultrasonic bath, plugging the end of the Erlenmeyer flask with cling film. After filtration of the macerate, 05 ml of the filtrate are collected and then evaporated in the dark. The residue obtained is dissolved in 10 mL of methanol and then passed through an ultrasonic bath for 10 minutes for homogenization. The methanolic solution is filtered again through a 0.45 µm Millipore filter and then stored in vials. Subsequently, 20 µL of each sample prepared under the preceding conditions are injected for chromatographic analysis according to the conditions.

Determination of minerals [15]

Determination of organic matter and ash

The method adopted is that often used for the determination of organic matter in sediments. It consists in calcining the sample at 450°C for 3 hours, (without pre-drying at 103 ± 2°C) the observed mass loss is attributed to the organic matter and therefore represents the mass percentage of organic matter. Then sample solutions were prepared.

Assay with atomic absorption spectrophotometer (AAS)

The wavelengths of the elements to be analyzed were first defined on the device. Then, the different readings of the calibration ranges allowed to establish the calibration curve reflecting the absorbance as a function of the concentration. Finally, the solutions containing ash are analyzed to determine the absorbance. Note that it is mandatory to pass the blank between the passage of two different solutions

Antioxidant activity (DPPH test) [16]

Principle

2,2 "-Diphenyl-1-picrylhydrazyl (DPPH) is a stable radical exhibiting specific absorption at 517 nm due to its purple color which disappears when reduced by a radical scavenger or an antioxidant. The DPPH test is based on the discoloration of the 2,2 "-diphenyl-1-picrylhydrazyl of violet color to 2,2'-diphenyl-1-picrylhydrazine of yellow color by plant extracts or standard (ascorbic acid) having an antioxidant activity.

Method

The test was carried out on the hydroethanolic extracts of the various leaves of *Annona muricata, Launaea taraxacifolia* and *Tridax procumbens* respectively. It consisted in preparing 1 mL of extract or standard (0.5-0.035 mg/mL) at different concentrations in 10 test tubes. Then 1 ml of the ethanolic solution of DPPH was added. The mixture was incubated for 30 minutes in the dark. The reading was taken with a spectrophotometer in the visible range at 517nm. The percentage of radical inhibition was determined by the following formula: Percentage of radical inhibition (%) = Absorbance of the solution of DPPH-Absorbance of the solution of DPPH in the presence of extract or standard/(Absorbance of the solution of DPPH) * 100.

The antioxidant power of the extracts was determined by calculating the concentration of extract or standard which inhibits by half the initial concentration of DPPH (IC50).

Results

Phytochemical analysis of the three plants

The extracts of the 3 plants together contain the following compounds in decreasing proportions: flavonoids, gallic tannins, leucoanthocyanins, mucilages, catechic tannins, anthocyanins, steroids, reducing compounds, alkaloids, triterpenoids, coumarins, cardiotonic heterosides (Table 1).

Compounds	*Launaea taraxacifolia*	*Tridax procumbens*	*Annona muricata*
Alkaloids	-	-	+
Catechic Tannins	++	-	-
Gallic Tannins	+	+	+
Flavonoids	++	++	-
Anthocyanins	+	+	-
Leucoanthocyanins	++	-	+
Quinonic derivatives	-	-	-
Saponosides	-	-	-
Triterpenoids	+	-	-
Steroids	+	+	-
Cyanogenic derivatives	-	-	-
Mucilages	+	+	+
Reducing compounds	-	+	+
Coumarins	-	-	+
Free anthracenics	-	-	-
Combined anthracenics: O-heterosides	-	-	-
Genin-reduced O-heterosides	-	-	-
Combined anthracenics: C-heterosides	-	-	-
Cardiotonic heterosides	-	-	+

Table 1: Summary of the phytochemical analysis of different plant extracts.

(+) : presence ; (++) : Abundance ; (-) : absence.

Vitamins content

The leaves of the 3 plants contain the following vitamins in decreasing concentrations per 100g: vitamin C: 534mg in *Tridax procumbens*, 450 mg in *Annona muricata* and 161 mg in *Launaea taraxacifolia*; vitamin E: 180mg in *Annona muricata;* 6,372 mg in *Tridax procumbens* and 0,168 mg in *Launaea taraxacifolia*; vitamin A: 8 mg in *Annona muricata*; 0,701 mg in *Launaea taraxacifolia* and 0,206 mg in *Tridax procumbens*; vitamin K1: 3,936 mg in *Tridax procumbens* and 1,021 mg in *Launaea taraxacifolia* (Table 2).

Plants	Average content in mg / 100g			
	Vitamine A	Vitamine E	Vitamine K1	Vitamine C
Launaea taraxacifolia	0,701	0,168	1,021	161
Tridax procumbens	0,206	6,372	3,936	534
Annona muricata	8	180	-	450

Table 2: Vitamin content of the leaves of the three plants.

Minerals content

The leaves of the 3 plants contain the following minerals in decreasing proportions per 100g: Iron: 3800 mg in *Annona muricata* and absent in the other two plants; calcium: 1934mg in *Annona muricata*, 827mg in *Tridax procumbens,* 257,7mg in *Launaea taraxacifolia*; potassium: 1639,7mg in *Tridax procumbens*, 1240,9mg in *Launaea taraxacifolia* and 324mg in *Annona muricata*; sodium: 928,4mg in *Tridax procumbens*, 929,8mg in *Launaea taraxacifolia* and 8,22mg in *Annona muricata*; Magnesium: 302mg in *Annona muricata*, 249mg in *Launaea taraxacifolia* and 235mg in *Tridax procumbens* (Table 3).

Minerals	Minerals content in mg/100 g		
	Launaea taraxacifolia	***Tridax procumbens***	***Annona muricata***
Calcium (Ca)	257,7	827	1934
Iron (Fe)	-	-	3800
Potassium (K)	1240,9	1639,7	324
Magnesium (Mg)	249	235	302
Sodium (Na)	929,8	928,4	8,228

Table 3: Mineral content of the leaves of the 3 plants.

Antioxidant activities of hydroethanolic extracts from the 3 plants

The antioxidant activities of hydroethanolic plant extracts were compared with that of ascorbic acid and gallic acid (reference antioxidants). The hydroethanolic extract of *Annona muricata* leaves exhibits anti-free radical activity characterized by an IC50 of 0.0091 mg/ml. The hydroethanolic extracts of the leaves of *Tridax procumbens* and *Launaea taraxacifolia* exhibit anti-radical activities characterized by IC50s of 0.17 mg/ml and 2.36 mg/ml respectively. These values are compared to the IC50 of ascorbic acid which is 0.00025mg/ml and that of gallic acid 0.038mg/ml.

Discussion

Role of oxidative stress and inflammation in the development of atherosclerosis

Several factors are responsible for the development of atherosclerosis: high blood pressure, high cholesterol (LDL), type 2 diabetes, smoking, etc. All these factors induce oxidative stress which constitutes, to varying degrees, the component of cellular and molecular events deleterious to vascular tissue, both in its structure and its functions [17]. Arterial hypertension, for example, activates the renin-angiotensin system, which induces oxidative stress via the activation of NAD (P) oxidase by the angiotensin 2 receptor AT1. This results in the production of superoxides which inactivate the nitric acid and causes vasodilation of blood vessels. High levels of bad cholesterol (LDL) in the blood cause oxidative stress due to the oxidation of low density lipoproteins, LDL, which leads to the formation of atheroma. The increased production of reactive oxygen species (ROS), and nitrogen (ERN) by arterial wall cells induces an inflammatory response and proliferation of smooth muscle cells, causing atherosclerotic lesion. Inflammation also plays a critical role in all stages of atherosclerosis, from the initiation and progression of lesions to clinical destabilization and the formation of vulnerable plaque [7].

The combination of the leaves of *Annona muricata, Tridax procumbens* and *Launaea taraxacifolia* and their antioxidant and anti-inflammatory activities

The three plants together are rich in flavonoids, tannins and also contain anthocyanins and triterpenoids. All of these compounds share antioxidant, anti-inflammatory and immunomodulatory properties [18,19]. The leaves of all three plants are also rich in vitamins C, E, A and K1. Vitamin E is an excellent antioxidant which protects cell membranes and helps neutralize free radicals

in the body [20]. In addition it regulates inflammation and immune system [21]. One of the important roles of vitamin C is its antioxidant effect which protects cells against damage inflicted by free radicals. Studies show that a diet rich in vitamin C (fruits and vegetables) is associated with a reduced risk of cardiovascular disease [22]. Vitamin C and E with their antioxidant activities could prevent atherosclerosis and cardiovascular diseases [23]. The hydroethanolic extracts of the leaves of *Annona muricata*, of *Tridax procumbens* and of *Launaea taraxacifolia* exhibit an anti-free radical activity characterized by the IC50 respectively equal to 0.009 mg/ml; 0.17mg/ml and 2mg/ml and these values are compared with that of vitamin C which is 0.00025 mg/ml and that of gallic acid which is 0.038 mg/ml. *Annona muricata* has greater anti-free radical power than *Tridax procumbens,* which has greater anti-free radical power than *Launaea taraxacifolia*. This antioxidant power of each plant is related to the presence of vitamin C, vitamin E and phenolic compounds in the plant and confirms the antioxidant activity of plants.

The combination of the three plants and their hypotensive and protective activities for the heart and blood vessels

All three plants also contain leucoanthocyanins, triterpenoids and cardiotonic glycosides. Leucoanthocyanins restore blood vessels, triterpenoids have vasodepressant, cardiotonic and antidysrhythmic effects [24]. Cardiotonic glycosides are characterized by their pharmacological properties of strengthening, slowing and regulating the heartbeat [25]. Vitamin K1 provided by *Tridax procumbens* may play a protective role against atherosclerosis and cardiovascular diseases. Indeed, a link has been established between vitamin K1 deficiency and an increased risk of arterial calcification. Researchers have also shown that taking vitamin K1 supplements slows the progression of this calcification or reduces the loss of elasticity in the arteries [26,27]. The three plants contain a significant amount of magnesium. Magnesium controls the entry and exit of calcium into cells, which allows muscle fibers to relax after exercise. The heart is a very important muscle, and having sufficient levels of magnesium has proven to protect against cardiovascular disease. It also helps maintain proper blood pressure, preventing hypertension [28,29]. *Launaea taraxacifolia* and *Tridax procumbens* are very rich in potassium. Potassium plays several roles in the body including regulating blood pressure; a diet rich in potassium lowers blood pressure and therefore reduces the risk of stroke and heart disease [30]. *Annona muricata* is rich in calcium and iron. A sufficient intake of calcium in accordance with the recommended nutritional intake would have a preventive effect against heart failure and diseases [31]. Iron is a component of hemoglobin inside red blood cells, which is used to supply various organs with oxygen. Iron supports the transport of oxygen necessary for optimal heart contraction. In a placebo-controlled study, iron-deficient heart failure patients, whether anemic or not, who received iron supplementation in injectable form, saw significant improvement in their heart failure symptoms and quality of life [32,33].

Conclusion

The leaves of *Annona muricata, Launaea taraxacifolia* and *Tridax procumbens* contain phenolic compounds (flavonoids and tannins), vitamins (C, E) which have antioxidant properties capable of neutralizing free radicals generated in the process of atherosclerosis. The minerals (potassium, magnesium, iron, calcium) contained in these plants could reduce blood pressure and promote the proper functioning of the heart muscle. Vitamin K1 and leucoanthocyanins also present in plants have a protective role for blood vessels. The mixture of these three plants therefore could represent an herbal medicine that could be used to reduce blood pressure and prevent the development of atherosclerosis and cardiovascular disease.

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